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Characterization of *Anaplasma marginale* Isolated from North American Bison

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Anaplasma marginale **(Rickettsiales: Anaplasmataceae), a tick-borne pathogen of cattle, is endemic in tropical and subtropical regions of the world. Although serologic tests have identified American bison, *Bison bison*, as being infected with *A. marginale*, the present study was undertaken to confirm *A. marginale* infection and to characterize isolates obtained from naturally infected bison in the United States and Canada. Major surface protein (MSP1a and MSP4) sequences of bison isolates were characterized in comparison with New World cattle isolates. Blood from one U.S. bison was inoculated into a susceptible, splenectomized calf, which developed acute anaplasmosis, demonstrating infectivity of this *A. marginale* bison isolate for cattle. The results of this study showed that these *A. marginale* isolates obtained from bison were similar to ones from naturally infected cattle.**

Anaplasma marginale (Rickettsiales: Anaplasmataceae) is a rickettsial pathogen that causes the disease anaplasmosis in cattle (reviewed by Kocan et al. 16). Feeding ticks effect biological transmission of this obligate intraerythrocytic organism, while mechanical transmission occurs when infected blood is transferred to susceptible cattle by biting flies or blood-contaminated fomites (reviewed by Ewing 13). Many geographic isolates of *A. marginale* which differ in biology, morphology, protein sequence and antigenic characteristics have been identified by the characterization of major surface protein 1a (MSP1a), which varies in sequence and molecular weight due to different numbers of tandem 28- or 29-amino-acid repeats (1; reviewed in reference 4).

MSPs involved in host-pathogen interactions may evolve more rapidly than other nuclear genes because of selective pressures exerted by host immune systems. Of the six *A. marginale* MSPs that have been identified and characterized, only three (MSP1a, MSP4, and MSP5) are encoded by single genes. Because these MSPs do not appear to undergo antigenic variation in cattle or ticks (2), they were considered to be more stable genetic markers for phylogenetic studies (4, 5, 8, 11, 14, 17). MSP1a, encoded by *msp1*α, has been reported to be an adhesin for bovine erythrocytes and tick cells and to be involved in adhesion, infection, and transmission of *A. marginale* by *Dermacentor* spp. ticks (6, 7, 10, 18, 19). Recent data support the existence of genetic heterogeneity in the structure of *msp1*α sequences recovered from infected cattle within a state (8, 11) and even in a single herd of cattle in areas of endemicity (11, 20), thus bringing into question the use of MSP1a sequences to identify geographic isolates of *A. marginale*. Single *msp1*α genotypes have been identified in individual cattle that were naturally or experimentally infected and sampled at different stages of infection (2, 20), a finding that could be explained by the exclusion of *A. marginale* isolates in previously infected cattle and ticks (9, 12). Furthermore, recent results provided evidence that *msp1*α is under positive selection pressure and suggested rapid evolution of *msp1*α sequences, resulting in dissemination of *A. marginale* genotypes by movement of cattle and maintenance of these genotypes in cattle populations through independent transmission events (11, 20). The specific function of MSP4 and MSP5 is not known. However, previous analysis of the *msp4* gene from *A. marginale* isolates demonstrated sufficient sequence variation to support its use in phylogeographic studies (5, 8, 11). In contrast to MSP4, MSP5 sequences are conserved between isolates of *A. marginale* and are not phylogenetically informative (25). Therefore, MSP5 has been used as a marker for the molecular diagnosis of *A. marginale* infection (15, 24).

American bison, *Bison bison*, have been reported to be infected with *A. marginale* on the basis of serologic surveys of naturally infected animals and to be susceptible to *A. marginale* in experimental infection studies (22, 26). However, *A. marginale* had not been isolated from naturally infected bison and characterized. The present study was undertaken to confirm *A. marginale* infection and to characterize isolates of *A. marginale* derived from naturally infected American bison from two widely separated geographic areas in the United States and Canada.

Ten farmed female bison (3 to 9 years old; average age, 4.8 years old) from a herd located on a single premise in central

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TABLE 1. Characterization of *A. marginale* infection in Canadian bison by serology and PCR

Sample no.	Sample identification	Anaplasmosis CFT titer	Anaplasmosis cELISA result (% inhibition)[b]	PCR result[a]		
				msp5	*msp4*	*msp1α*
1	693-1	1/20	71.28	Positive	Positive	Negative
2	693-2	1/40	86.99	Positive	Negative	Negative
3	693-3	1/40	73.46	Positive	Positive	Negative
4	693-4	1/5	90.78	Positive	Positive	Negative
5	693-5	1/20	81.65	Positive	Positive	Negative
6	693-6	1/5	63.77	Positive	Positive	Positive
7	693-7	1/80	91.17	Positive	Positive	Negative
8	693-8	1/10	55.62	Positive	Positive	Negative
9	693-9	1/20	75.76	Positive	Positive	Negative
10	693-10	1/10	60.57	Positive	Positive	Negative

[a] A positive PCR result indicates that the appropriate-sized fragment was visualized by UV transillumination of DNA products for the corresponding sample on agarose gels stained with ethidium bromide. A negative PCR result indicates that no amplified products were detectable for the corresponding sample. PCR for *msp5* was performed on a different blood sample (collected 6 weeks previously) than the sample used for performing the CFT, cELISA, and PCRs for *msp1α* and *msp4*.
[b] cELISA samples with a percent inhibition of >40% were positive.

Saskatchewan, Canada, and 50 female bison (3 to 13 years old; average age, 10.7 years old) that were being culled from a bison herd maintained by Nature Conservancy at the Tallgrass Prairie Preserve near Pawhuska, Okla., were used for this study. Blood samples and sera were collected from the bison for serologic and molecular biology studies. Blood samples were collected from Canadian bison prior to euthanasia of the animals for regulatory purposes. Erythrocytes from blood samples collected from U.S. bison were washed three times in phosphate-buffered saline, resuspended 1:1 with phosphate-buffered saline and 10% dimethyl sulfoxide, and frozen in liquid nitrogen to prepare blood stabilates.

Identification of bison naturally infected with *A. marginale*. Infection of bison with *A. marginale* was detected by using the anaplasmosis complement fixation test (CFT) (3), the MSP5 competitive-inhibition enzyme-linked immunosorbent assay (cELISA) (15), and/or nested PCR for the *msp5* gene (24).

The CFT was performed as described by the Official Protocol at the Canadian Food Inspection Agency Lethbridge Laboratory (3). The antigen used in the CFT was derived from erythrocytes of splenectomized calves originally infected with *A. marginale* stabilate obtained from National Veterinary Service Laboratory, U.S. Department of Agriculture, Ames, Iowa. This antigen was used to test doubling dilutions of bison serum in the presence of guinea pig serum. Sheep erythrocytes sensitized with rabbit antibody were used as the indicator system, and hemolysis was assessed visually. The anaplasmosis cELISA was performed with an anaplasma antibody test kit from Veterinary Medical Research and Development, Inc. (Pullman, Wash.), following the manufacturer's instructions. This assay specifically detects the presence of serum antibodies that target MSP5 of *Anaplasma* spp. (15).

For the *msp5* nested PCR, DNA was extracted from 300 μl of Canadian bison blood collected in EDTA-treated tubes by using the Puregene kit (Gentra Systems, Inc.) and following the manufacturer's instructions for blood. PCR for the *msp5* gene was performed on a blood sample collected 6 weeks before the sample used for the CFT, cELISA, and PCRs for *msp1α* and *msp4*. The nested PCR was performed on the extracted DNA by using primers specific for the *msp5* gene as described by Torioni de Echaide et al. (24). Briefly, *msp5* was amplified by using forward (5′-GCATAGCCTCCGCGTCCTTC-3′) and reverse (5′-TCCTCGCCTTGGCCCTCAGA-3′) primers for the first (primary) PCR and the forward primer 5′-TACACGTGCCCTACCGAGTTA-3′ and the reverse primer used for the primary reaction for the secondary (semi-nested) PCR. Both reactions were performed under the same cycling conditions with a GeneAmp 2400 system (Applied Biosystems Inc.) beginning with a hot start for 3 min at 95°C followed by 35 cycles with denaturation for 30 s at 95°C, annealing for 58 s at 65°C, and extension for 30 s at 72°C and a final extension for 10 min at 72°C. The product (1 μl) of the primary PCR was then run under the same conditions for an additional 35 cycles with the appropriate primers for the secondary PCR. Control reactions were performed with the same procedures but without DNA, to rule out contamination of the PCR. Amplification products were analyzed by gel electrophoresis using 1.2% agarose containing ethidium bromide and visualized by UV transillumination. Based on the *msp5* nucleotide sequence of *A. marginale* (25) (GenBank accession number M93392), a 457-bp band is expected after the primary PCR and a 344-bp band is expected after nested PCR.

Samples from all 10 Canadian bison presented titers ranging from 1/5 to 1/80 in the anaplasmosis CFT and percent inhibition values greater than 40% in the anaplasmosis cELISA, indicating the presence of antibodies to the *A. marginale* MSP5 (Table 1). The presence of *msp5* gene product was detected by nested PCR in DNA extracted from each of the 10 blood samples from Canadian bison (Table 1). Blood samples collected from 42 of the 50 U.S. bison proved to be positive for *A. marginale* by the CFT and/or cELISA (Table 2). Stabilate from U.S. bison 44, which was positive by *A. marginale*-specific cELISA and CFT serology (Table 2), was tested for infectivity for cattle. The frozen stabilate was allowed to thaw and then immediately inoculated intravenously into a susceptible, splenectomized calf, calf PA488. Inoculation of stabilate from U.S. bison 44 caused anaplasmosis in calf PA488, which became infected with *A. marginale* (prepatent period of 33 days, reduction of packed cell volume of 46.9%, and a peak percent infected erythrocytes of 62.2%), thus demonstrating infectivity of this bison isolate for cattle.

Molecular characterization of bison *A. marginale*. Molecular characterization of bison *A. marginale* was done with *msp4* and *msp1α* sequences. *A. marginale* DNA was extracted from 0.5 ml

TABLE 2. Characterization of *A. marginale* infection in U.S. bison by cELISA and CFT serology

Sample	Anaplasmosis CFT titer[a]	Anaplasmosis cELISA result (% inhibition)[b]
1	<1/5	**71.81**
2	<1/5	**49.20**
3	<1/5	21.54
4	<1/5	**78.72**
5	1/5	**80.59**
6	<1/5	**39.63**
7	<1/5	**84.04**
8	<1/5	**44.95**
9	<1/5	**82.45**
10	1/40*	**83.24**
11	<1/5	**78.72**
12	1/40*	**84.04**
13	<1/5	**80.59**
14	<1/5	**84.84**
15	<1/5	25.69
16	<1/5	**43.06**
17	<1/5	**51.39**
18	<1/5	20.14
19	AC	22.22
20	AC	**38.54**
21	<1/5	**46.18**
22	<1/5	**81.60**
23	<1/5	28.13
24	<1/5	**34.03**
25	1/5	**82.99**
26	ND	**80.90**
27	<1/5	15.63
28	1/40	**77.08**
29	<1/5	**79.86**
30	<1/5	**85.42**
31	<1/5	**35.42**
32	<1/5	**69.44**
33	<1/5	**83.68**
34	<1/5	**76.04**
35	<1/5	27.78
36	1/5	**84.72**
37	<1/5	**35.42**
38	<1/5	**64.58**
39	<1/5	21.88
40	AC	**32.29**
41	<1/5	**86.46**
42	1/10	**77.08**
43	<1/5	**73.96**
44	1/20	**82.64**
45	<1/5	**74.31**
46	<1/5	**79.17**
47	1/40	**85.42**
48	<1/5	**83.33**
49	1/10	**87.15**
50	<1/5	**38.89**

[a] *, atypical reaction; AC, anticomplementary reaction; ND, not done.
[b] cELISA samples with a percent inhibition of >30% (bold) were positive.

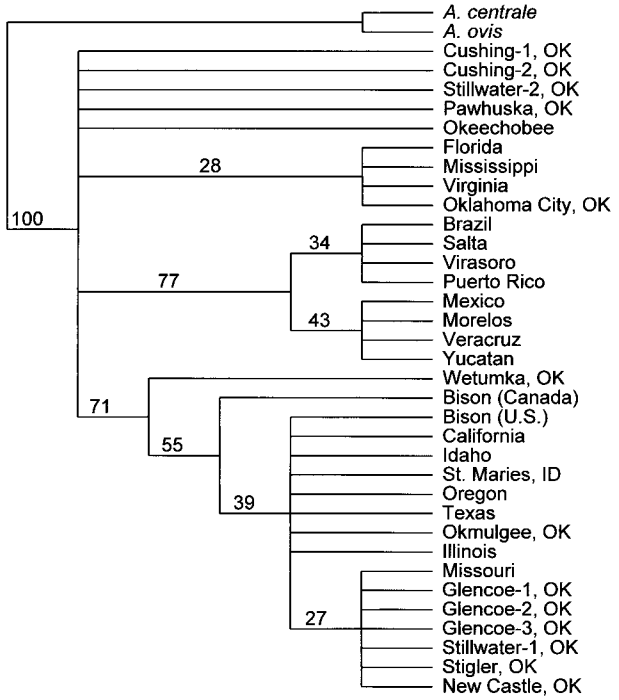

FIG. 1. Phylogenetic affinities among isolates of *A. marginale* based on unweighted maximum parsimony analysis of character state changes of *msp4*. Topology of 50% majority rule consensus tree of 59 equally parsimonious trees of 193 steps (consistency index = 0.7629; retention index = 0.9205) is shown. Numbers above branches are percentages of 500 bootstrap iterations in which each clade was detected.

of blood collected in EDTA-treated tubes with TRI reagent (Sigma) as previously reported (5). The *msp1*α gene was amplified from 1 μl (1 to 10 ng) of DNA by PCR using 10 pmol each of primers MSP1aP (5′GCATTACAACGCAACGCTTGAG3′) and MSP1a3 (5′GCTTTACGCCGCCGCCTGCGCC3′) in a 50-μl volume (1.5 mM $MgSO_4$, 0.2 mM deoxynucleoside triphosphate, 1× avian myeloblastosis virus/*Tfl* reaction buffer, 5 U of *Tfl* DNA polymerase) employing the Access reverse transcriptase PCR system (Promega, Madison, Wis.). Reactions were performed in an automated DNA thermal cycler (Mastercycler personal; Eppendorf, Westbury, N.Y.) for 35 cycles. After an initial denaturation step of 30 s at 94°C, each cycle consisted of a denaturing step of 30 s at 94°C and an annealing-extension step of 2.5 min at 68°C. The program ended by storing the reaction mixtures at 4°C. The *msp4* gene was amplified as described above but with oligonucleotides MSP45 (5′GGGAGCTCCTATGAATTACAGAGAATTGTTTAC3′) and MSP43 (5′CCGGATCCTTAGCTGAACAGGAATCTTGC3′) and a PCR profile of a denaturing step of 30 s at 94°C, annealing for 30 s at 60°C, and an extension step of 1 min at 68°C. PCR products were electrophoresed on 1% agarose gels stained with ethidium bromide to check the size of amplified fragments by comparison to a DNA molecular weight marker (1 Kb Plus DNA ladder; Promega).

Amplified fragments were resin purified (Wizard; Promega) and cloned into pGEM-T vector (Promega) (*msp1*α) or used directly (*msp4*) for sequencing both strands by double-stranded dye-termination cycle sequencing (Core Sequencing Facility, Department of Biochemistry and Molecular Biology, Noble Research Center, Oklahoma State University). At least two clones were sequenced from each PCR.

Multiple sequence alignment was performed with the program AlignX (Vector NTI Suite V 5.5; InforMax, Bethesda, Md.) with an engine based on the Clustal W algorithm (23). Nucleotides were coded as unordered, discrete characters with five possible character states—A, C, G, T, or N—and gaps

TABLE 3. Sequences of MSP1a tandem repeats in bison and U.S. cattle isolates of *A. marginale*[a]

Repeat form	Encoded sequence
A	DDSSSASGQQQESSVSSQSE-ASTSSQLG-
B	A*****G**********DQ*********
C	A*****G**********GQ*********
D	A**************************G
E	A***************************
F	T*****************GQ*********
G	******************GQ******S**
H	T*****************GQ******S**
I	******************GQ*********
J	A***L*G**********DQ*********
K	A*G***G**********DQ*********
L	AG****D**********DQ*********
M	A*****************GQ*********
N	T*****************DQ*********
O	----**G**********DQ*********
P	T**********G****GQ**H*A*S**
Q	A*****************DQ*********
R	A*****G***H*******DQ*******W*
S	A*G***G**********DQ*********
T	AG****G**********DQ*********
U	******************DQ*********
V	A*****G***-*******DQ*********
W	T*****************GQ******SR*

[a] The one-letter amino acid code is used to depict the different sequences found in MSP1a repeats. Repeat forms A to J were designated according to the work of Palmer et al. (20). Asterisks indicate identical amino acids; hyphens indicate deletions and insertions.

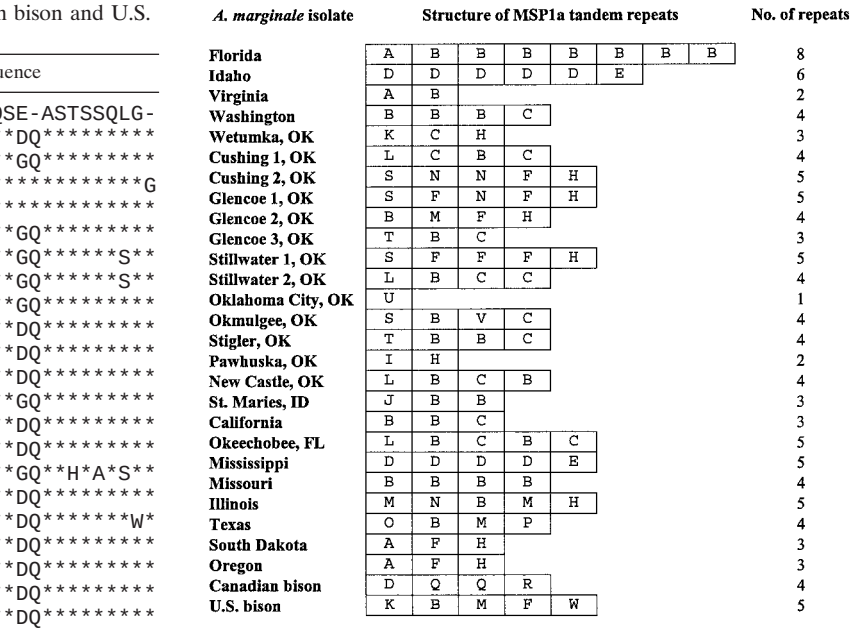

FIG. 2. Structure of the MSP1a repeat region for bison and U.S. cattle isolates of *A. marginale* using the repeat forms in Table 3.

were coded as missing data. Maximum parsimony analyses were conducted with equal weights for all characters and substitutions and with heuristic searches with 25 random additions of input taxa and tree bisection-reconnection branch swapping using PAUP 4.0b4a (21). Stability or accuracy of inferred topologies was assessed via bootstrap analysis of 500 iterations of heuristic searches with 25 random additions of input taxa and tree bisection-reconnection branch swapping. Character state changes were polarized by designating *Anaplasma centrale* (AF428090) and *Anaplasma ovis* (AF393742) as outgroups.

The *msp4* coding region was amplified by PCR in all Canadian blood samples except for sample 2 (Table 1). No amplification signal was detected in blood samples from U.S. bison. The *msp4* sequence of the U.S. bison *A. marginale* was amplified from a blood sample of calf PA488 infected with a stabilate from U.S. bison 44 (peak percent infected erythrocytes = 17.8% and packed-cell volume = 31.5%). All nine Canadian bison-derived *msp4* sequences were identical, with a characteristic silent nucleotide change of A to G at position 81 with respect to the translation initiation codon, not found in the sequence of the U.S. bison *A. marginale* or in previously sequenced *A. marginale* isolates from cattle. The *A. marginale msp4* coding region was used for sequence alignment and phylogenetic analysis. *A. marginale* isolates from naturally infected cattle that were reported previously were used for phylogenetic comparison (5, 8, 10, 11), except for an isolate from Puerto Rico for which *msp1*α (AY191826) and *msp4* (AY191827) sequences were obtained from GenBank. *A. marginale* isolates included 24 isolates from the United States, named according to the U.S. state in which they were isolated, except for isolates from Oklahoma, which were named after the city from which they were obtained (8, 11). Also included were eight isolates from Latin America (four [Mexico, Yucatan, Morelos, and Veracruz] from Mexico; two [Salta and Virasoro] from Argentina, one [Brazil] from Brazil, and one from Puerto Rico) (8).

Maximum parsimony analysis of *msp4* sequences of *A. marginale* isolates from bison and cattle from the New World resulted in 59 equally parsimonious trees of 193 steps (consistency index = 0.7629; retention index = 0.9205). Strong support was detected for a Latin American clade and a clade of isolates from the west-central United States which contained the bison isolates (Fig. 1).

The region for the *msp1*α gene was amplified from Canadian bison sample 6 (Table 1) and the *A. marginale* isolated from U.S. bison 44 after inoculation into calf PA488. The sequences demonstrated the presence of four and five tandem repeats, respectively, in the amino-terminal portion of the protein as described for other *A. marginale* isolates (Table 3; Fig. 2). However, the MSP1a sequence of bison isolates of *A. marginale* had three repeat forms, Q, R, and W, that are not present in any other sequenced isolate of *A. marginale* (Table 3; Fig. 2).

As demonstrated in previous studies (5, 8, 11), *msp4* sequences provided phylogeographic patterns for *A. marginale* isolates on a broad geographic scale. The *msp4* sequence characters subdivided the U.S. cattle isolates into southeastern-central (isolates from Florida, Mississippi, Virginia, and Oklahoma) and west-central (isolates from California, Idaho, Illinois, Oregon, Missouri, Texas, and Oklahoma) clades. The *msp4* sequence for bison isolates grouped together with U.S. west-central cattle isolates of *A. marginale*. However, as demonstrated for isolates from Oklahoma, extensive cattle movement could affect the geographic distribution of *A. marginale* isolates (11). The *msp4* sequences from the 10 bison isolates from Canada were identical to each other but different from the sequence of the U.S. bison isolate, which was found to be identical to the sequence of cattle isolates from the U.S. There-

fore, the phylogeographic positioning of the bison *A. marginale* should be considered provisional.

The *msp1*α and *msp4* genes were not amplified directly from U.S. bison blood. Furthermore, the *msp1*α gene was amplified from only one Canadian bison blood sample. PCR for *msp5* was performed on a blood sample collected 6 weeks before the sample used for performing the PCRs for the *msp1*α and *msp4* genes. Therefore, the differences in the detection of *msp4*, *msp5*, and *msp1*α in bison blood samples could be due to differences in the level of rickettsemia between the blood samples or to differences in the sensitivity of the PCR assays (9, 24). However, we routinely amplify *msp1*α from bovine blood with rickettsemia equal or higher than 1% infected erythrocytes. Therefore, these results suggest that the infection levels in most bison blood samples were below 1% infected erythrocytes, which is not unusual for persistently infected carrier animals. However, when infected blood from the U.S. bison was inoculated into a susceptible calf, *A. marginale* established infection in a manner similar to that observed with cattle *A. marginale* isolates.

The results reported herein confirm that bison can be naturally infected with *A. marginale*. Sequence information for *A. marginale* isolates from bison in Canada and the U.S. suggests that these isolates are closely related to isolates of *A. marginale* from U.S. cattle.

Nucleotide sequence accession numbers. The GenBank accession numbers for the *msp1*α and *msp4* sequences of *A. marginale* bison isolates are AY253141 to AY253144.

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ADDENDUM IN PROOF

After the manuscript was submitted, we demonstrated that *Dermacentor variabilis* male ticks, after feeding on the U.S. bison no. 44 *A. marginale* isolate-infected calf PA488, transmitted the bison *A. marginale* to another susceptible bovine calf.

REFERENCES

1. **Allred, D. R., T. C. McGuire, G. H. Palmer, S. R. Leib, T. M. Harkins, T. F. McElwain, and A. F. Barbet.** 1990. Molecular basis for surface antigen size polymorphisms and conservation of a neutralization-sensitive epitope in *Anaplasma marginale*. Proc. Natl. Acad. Sci. USA **87:**3220–3224.
2. **Bowie, M. V., J. de la Fuente, K. M. Kocan, E. F. Blouin, and A. F. Barbet.** 2002. Conservation of major surface protein 1 genes of the ehrlichial pathogen *Anaplasma marginale* during cyclic transmission between ticks and cattle. Gene **282:**95–102.
3. **Canadian Food Inspection Agency.** 2002. Microwarm complement fixation test (CFT) for the detection of serum antibody to *Anaplasma marginale*. Official Protocol of the CFIA Lethbridge Laboratory, version 1.1. Canadian Food Inspection Agency, Ottawa, Canada.
4. **de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, S. D. Rodríguez, M. A. García, and K. M. Kocan.** 2001. Evolution and function of tandem repeats in the major surface protein 1a of the ehrlichial pathogen *Anaplasma marginale*. Anim. Health Res. Rev. **2:**163–173.
5. **de la Fuente, J., R. A. Van Den Bussche, and K. M. Kocan.** 2001. Molecular phylogeny and biogeography of North American isolates of *Anaplasma marginale* (Rickettsiaceae: Ehrlichieae). Vet. Parasitol. **97:**65–76.
6. **de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, and K. M. Kocan.** 2001. Differential adhesion of major surface proteins 1a and 1b of the ehrlichial cattle pathogen *Anaplasma marginale* to bovine erythrocytes and tick cells. Int. J. Parasitol. **31:**145–153.
7. **de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, and K. M. Kocan.** 2001. Major surface protein 1a effects tick infection and transmission of the ehrlichial pathogen *Anaplasma marginale*. Int. J. Parasitol. **31:**1705–1714.
8. **de la Fuente. J., R. A. Van Den Bussche, J. C. Garcia-Garcia, S. D. Rodriquez, M. A. Garcia, A. A. Guglielmone, A. J. Mangold, L. M. Friche Passos, M. F. Barbosa Ribeiro, E. F. Blouin, and K. M. Kocan.** 2002. Phylogeography of New World isolates of *Anaplasma marginale* (Rickettsiaceae: Anaplasmataceae) based on major surface protein sequences. Vet. Microbiol. **88:**275–285.
9. **de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, J. T. Saliki, and K. M. Kocan.** 2002. Infection of tick cells and bovine erythrocytes with one genotype of the intracellular ehrlichia *Anaplasma marginale* excludes infection with other genotypes. Clin. Diagn. Lab. Immunol. **9:**658–668.
10. **de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, and K. M. Kocan.** 2003. Characterization of the functional domain of major surface protein 1a involved in adhesion of the rickettsia *Anaplasma marginale* to host cells. Vet. Microbiol. **91:**265–283.
11. **de la Fuente, J., R. A. Van Den Bussche, T. M. Prado, and K. M. Kocan.** 2003. *Anaplasma marginale* major surface protein 1α genotypes evolved under positive selection pressure but are not a marker for geographic isolates. J. Clin. Microbiol. **41:**1609–1616.
12. **de la Fuente, J., E. F. Blouin, and K. M. Kocan.** 2003. Infection exclusion of the rickettsial pathogen *Anaplasma marginale* in the tick vector *Dermacentor variabilis*. Clin. Diagn. Lab. Immunol. **10:**182–184.
13. **Ewing, S. A.** 1981. Transmission of *Anaplasma marginale* by arthropods, p. 395–423. *In* Proceedings of the 7th National Anaplasmosis Conference. Mississippi State University, Mississippi State.
14. **Kano, F. S., O. Vidotto, R. C. Pacheco, and M. C. Vidotto.** 2002. Antigenic characterization of *Anaplasma marginale* isolates from different regions of Brazil. Vet. Microbiol. **87:**131–138.
15. **Knowles, D. P., S. Torioni de Echaide, G. E. Palmer, T. C. McGuire, D. Stiller, and T. F. McElwain.** 1996. Antibody against an *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. J. Clin. Microbiol. **34:**2225–2230.
16. **Kocan, K. M., E. F. Blouin, and A. F. Barbet.** 2000. Anaplasmosis control. Past, present and future. Ann. N. Y. Acad. Sci. **916:**501–509.
17. **Lew, A. E., R. E. Bock, C. M. Minchin, and S. Masaka.** 2002. A msp1 alpha polymerase chain reaction assay for specific detection and differentiation of *Anaplasma marginale* isolates. Vet. Microbiol. **86:**325–335.
18. **McGarey, D. J., and D. R. Allred.** 1994. Characterization of hemagglutinating components on the *Anaplasma marginale* initial body surface and identification of possible adhesins. Infect. Immun. **62:**4587–4593.
19. **McGarey, D. J., A. F. Barbet, G. H. Palmer, T. C. McGuire, and D. R. Allred.** 1994. Putative adhesins of *Anaplasma marginale*: major surface polypeptides 1a and 1b. Infect. Immun. **62:**4594–4601.
20. **Palmer, G. H., F. R. Rurangirwa, and T. F. McElwain.** 2001. Strain composition of the ehrlichia *Anaplasma marginale* within persistently infected cattle, a mammalian reservoir for tick transmission. J. Clin. Microbiol. **39:**631–635.
21. **Swofford, D. L.** 2000. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.02b. Sinauer Associates, Inc., Sunderland, Mass.
22. **Taylor, S. K., V. M. Lane, D. L. Hunter, K. G. Eyre, S. Frye, and M. R. Johnson.** 1997. Serologic survey for infectious pathogens in free-ranging American bison. J. Wildl. Dis. **33:**308–311.
23. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
24. **Torioni de Echaide, S., D. P. Knowles, T. C. McGuire, G. H. Palmer, C. E. Suarez, and T. F. McElwain.** 1998. Detection of cattle naturally infected with *Anaplasma marginale* in a region of endemicity by nested PCR and a competitive enzyme-linked immunosorbent assay with recombinant major surface protein 5. J. Clin. Microbiol. **36:**777–782.
25. **Visser, E. S., T. C. McGuire, G. H. Palmer, W. C. Davis, V. Shkap, E. Pipano, and D. P. Knowles, Jr.** 1992. The *Anaplasma marginale msp5* gene encodes a 19-kilodalton protein conserved in all recognized *Anaplasma* species. Infect. Immun. **60:**5139–5144.
26. **Zaugg, J. L., and K. L. Kuttler.** 1985. *Anaplasma marginale* infections in American bison: experimental infection and serologic study. Am. J. Vet. Res. **46:**438–441.